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TITLE: LEPTOSPIROSIS, SCRUB TYPHUS AND COLORADO TICK FEVER
LIKE DISEASE IN KOREA

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Leptospirosis, scrub typhus and Colorado Tick Fever-like disease are all febrile diseases found in Korea which have a clinical presentation similar to that of Korean hemorrhagic fever. These diseases have all been shown to be of great military medical importance, and all have rodents as reservoirs, so it is natural to study them as a group. To facilitate research on these diseases, a new medical research laboratory, USAMRU-ROK, was set up in Seoul, Korea in the autumn of 1988. Construction and renovation of the new laboratory facilities were complete by the summer of 1990. Well before that time, starting in April, 1989, laboratory staff were hired and received extensive training both in Korea and in the United States and Japan. Actual research began in the fall of 1989, when this training was well underway, and only a year after the first members of the research team arrived in Korea. Current research projects include a study of farming practices throughout Korea; a case control study of risk factors in Korean soldiers; a country-wide survey of rodent reservoirs; a longitudinal ecological study of the principal reservoir, apodemus					
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agrarius; taxonomic studies of Apodemus species; and development of new diagnostic tests. In addition, the new laboratory provides support to U.S. forces throughout the Pacific theater.

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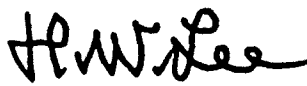
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TABLE OF CONTENTS

INTRODUCTION.....	1
A. Background.....	1
B. Hypotheses and Previous Work.....	1
C. Methods of Approach.....	2
1. General.....	2
2. Seroepidemiological Surveys of Leptospirosis, Scrub Typhus and Hantavirus in Korea	3
3. Identification of Specific Risk Factors	4
MATERIALS AND METHODS.....	4
A. Epidemiology.....	4
1. Farming Study.....	4
2. Case Control Study of Risk Factors.....	5
3. Blood specimens from civilian hospitals.....	6
4. Serosurvey of ROK Army Personnel.....	6
B. Field Ecology and Microbiology.....	6
1. Survey Area.....	6
2. Collection of Field Mammals.....	7
3. Specimens from Domestic Animals for Isolation of Leptospira.....	7
4. Specimens from Patients.....	7
5. Virus Isolation.....	7
6. Leptospiral Isolation.....	7
7. Rickettsial Isolation.....	8
8. Serological Tests for Leptospiral Antibodies.....	8
9. Serological Tests for Scrub Typhus Antibodies.....	8
10. Serological Tests for Murine Typhus and Spotted Fever Antibodies.....	8
11. Serological Tests for CTF like Agent.....	8
RESULTS.....	8
A. Laboratory Setup.....	8
B. Time Constraints.....	9
C. Epidemiology.....	9
1. Farming Study.....	9
2. Case Control Study of Risk Factors.....	11
3. Diagnostic Serology on Specimens Submitted by Civilian Hospitals in Korea in 1990.....	12
D. Field Ecology.....	13
1. Scrub and Murine Typhus.....	13
2. Leptospirosis.....	13
3. Colorado Tick Fever.....	13
E. Microbiology.....	13
1. General.....	13
2. Microbiological Support of the Field Ecology Department.....	14
3. Microbiological Support of the Epidemiology Department.....	14
4. Microbiological Support of U.S. Forces in the Pacific.....	14

CONCLUSIONS.....	15
REFERENCES.....	15
APPENDIX	
Figure 1. Geozoological Survey	16
Figure 2. USAMRU-ROK History	17
Table 1. Demographics of Leptospirosis Patients* Identified at the Capital Armed Forces General Hospital in Seoul, Korea, 10/89 to 10/90	18
Table 2. Demographics of Scrub Typhus Patients Identified at the Capital Armed Forces General Hospital in Seoul, Korea, 10/89 to 10/90	19
Table 3. Total Number of Patients with Hemorrhagic Fever with Renal Syndrome (HFRS), Murine Typhus, Scrub Typhus, Spotted Fever Group (SFG) Rickettsiosis and Leptospirosis Diagnosed Serologically Among Civilian Suspect Hemorrhagic Fever Patients in Korea, 1990	20
Table 4. Geographical Distribution of HFRS, Murine Typhus, Scrub Typhus, Spotted Fever Group (SFG) Rickettsiosis and Leptospirosis Among Civilian Suspect Hemorrhagic Fever Patients in Korea, 1990	21
Table 5. Monthly Incidence of HFRS, Murine Typhus, Scrub Typhus, Spotted Fever Group (SFG) Rickettsiosis and Leptospirosis Among Civilian Suspect Hemorrhagic Fever Patients in Korea, 1990	22
Table 6. Results of Mammalian Serology Tests Conducted by the Microbio- logy Department in Support of USAMRU-ROK Ecology Studies.	23
Table 7. Results of Mammalian Leptospirosis Cultures Conducted by the Microbiology Department in Support of USAMRU-ROK Ecology Studies.	24
Table 8. Results of Serological Tests Performed on Soldiers Hospitalized at the Capital Armed Forces General Hospital (6 Nov 1989 to Oct 1990) ...	25

INTRODUCTION

A. Background

The changing ecology of South Korea since the end of hostilities in 1954, including reforestation of much of the country, changes in farming practices, and increased rural exposure for some segments of both civilian and military populations, have led to increased risk of infection by various pathogens either directly or indirectly maintained by rodent hosts. Recent studies by H.W. Lee and colleagues have established the importance of hantaviruses (Hantaan, Seoul, and related viruses) in the causation of widespread human disease in Korea, as well as elsewhere in Asia. Intensive investigations in South Korea have further documented the presence and growing significance of other rodent-borne pathogens, especially leptospirosis and scrub typhus, which heretofore have not been recognized as major health problems. Investigation currently funded by USAMRDC under grant DAMD-17-86-G-6011 entitled "Hemorrhagic fever with renal syndrome (HFRS)" have allowed us to identify leptospirosis, scrub typhus, and perhaps Colorado tick fever or a closely related virus, as important causes of human disease which on initial clinical diagnosis have been confused with HFRS.

Historically leptospirosis and scrub typhus have both been important human pathogens of proven importance to the military. They are, however, diseases which are most common in developing countries of the world where research budgets are slight and qualified investigators rare. Consequently, these diseases have in general not benefited from the current revolution in biotechnology, nor has our understanding of their epidemiological characteristics progressed significantly over the past decades.

B. Hypotheses and Previous Work

1. Leptospirosis is an important cause of human disease in South Korea. Studies conducted by H.W. Lee and MAJ Ross Graham have shown that a significant proportion of human cases initially diagnosed as Korean hemorrhagic fever (HFRS due to Hantaan virus infection) were in fact due to leptospirosis. The extent of the human disease burden due to leptospirosis in Korea is presently unknown, due in part to the absence of careful scientific investigations in this disease, and to the absence of convenient diagnostic tests to rapidly identify acute infections. MAJ Graham has developed a sensitive, specific assay to rapidly diagnose acute leptospirosis infections. Similar assays have also been developed which will be useful in serosurveys to establish the prevalence of specific antibodies to leptospiral antigens. Field testing has shown that they will detect antibodies specific to the wide variety of pathogenic serovars known to exist worldwide. Studies in Korea indicate that these assays function well in human pathogenic

serovars known to occur in Korea. Consequently, modern, efficient serological tools are currently available to initiate the study of the objectives outlined in support of this hypothesis.

2. Scrub typhus is an important cause of human morbidity and mortality among both civilian and military populations in Korea. *R. tsutsugamushi* was isolated from field mice during the Korean Conflict; however, little has been done to investigate the epidemiological characteristics of this disease. Changes in the ecological conditions in Korea since the Korea Conflict and modifications in agricultural practices suggest that the current disease ecology may be quite different from that observed during investigations made during and after the Korea Conflict. This suggests that much remains to be learned regarding this important disease. Recent serosurvey results indicate that antibody prevalence rates to scrub typhus, as well as to other rickettsial pathogens, are quite high among some populations in Korea, including military units. As seen with leptospirosis, some patients clinically diagnosed as Korean hemorrhagic fever were in fact suffering from scrub typhus. These results suggest that scrub typhus is an important human pathogen of special concern to the military in Korea, and justify our proposed investigations and interest.
3. Colorado tick fever (CTF) or a closely related virus is involved in human diseases in the ROK. Colorado tick fever has never been recognized as a human pathogen in Asia; however, the studies mentioned above which examined Hantaan seronegative patients with a clinical diagnosis of Korean hemorrhagic fever revealed many sera contained antibodies specific for a virus identical or closely related to CTF. These observations have been confirmed by Dr. C. Calisher of CDC, Fort Collins in virus neutralization tests. This would suggest that perhaps a heretofore unrecognized viral pathogen exists in Korea which may be responsible for a portion of the non-hantaviral clinical disease encountered. Although the CTF virus hypothesis requires verification and may not withstand careful scientific scrutiny, the fact that a significant population with hemorrhagic fever with renal syndrome-like disease still exists annually without any known etiologic diagnosis clearly justifies further investigation of this disease.

C. Methods of Approach

1. General

Leptospira, scrub typhus and a Colorado tick fever-like agent are known to occur in Korea but little is known about serovars or serotypes present, the geographical distribution, or the ecological conditions. We propose to conduct serological surveys in humans

from different geographical areas in Korea to determine the prevalence and distribution of these three diseases. Since domestic animals play an important role in the epidemiology of leptospirosis in humans, serological surveys will also be done in bovine and swine populations to assess the distribution, serotypes and prevalence of leptospiral antibodies within these species. *Leptospira* can be found in the kidneys of infected animals for up to one year and attempts will be made to isolate leptospira from the kidneys of animals in slaughter houses throughout Korea. This will also provide more relevant serovars to be included in the microagglutination test for leptospiral antibodies in both humans and animals. Attempts will also be made to isolate leptospira, scrub typhus and any viral agent from acutely ill patients. Sera will also be collected in an attempt to diagnose the disease in those cases where isolation is not successful. This should be helpful in characterizing the clinical course of these diseases. Since rodents play an important role in all three of these diseases, studies of the species involved in maintenance and transmission, ecology and distribution are relevant. Serological and isolation attempts will be conducted in several areas within Korea. Since mites and ticks are also an important part of the disease these will also be collected when the rodents are trapped and processed for rickettsial and/or virus isolation.

The availability of clinical material from human clinical specimens and field samples from rodents, domestic animals and arthropods provide relevant specimens to identify target molecules (i.e., antigen, IgM and IgG) for new rapid diagnostic procedures. USAMRIID is currently involved in developing new procedures and as soon as these become available they will be fielded in Korea.

2. Seroepidemiological Surveys of Leptospirosis, Scrub Typhus and Hantavirus in Korea

While some data on the geographic distribution and relative frequency of these diseases in Korea is available, there are currently no accurate quantitative estimates of incidence and prevalence, either for the country as a whole or for specific areas within the country. Most of the data have been the result of hospital-based studies, and the number of cases found cannot be related to specific denominator populations. We propose to conduct cross-sectional surveys of selected rural populations throughout the country. Representative areas will be selected, and then a random sample of the population, including all age groups and both sexes, will be enrolled in the study. Demographic, occupational, and behavioral data will be collected and blood for serological testing will be drawn. This study should provide the following information:

(a) Prevalence of antibodies to each of the infectious agents in the total population.

(b) Prevalence of antibodies in subgroups of the population, enabling inferences to be made regarding age of antibody acquisition, relative risks of various occupations, etc.

In addition to cross-sectional studies of local civilian populations, we plan to do prospective cohort studies of Hantavirus, leptospirosis and rickettsial infections in the ROK Army and the U.S. Army. Army units which are stationed in areas of suspected high transmission based on previous studies will be identified and from these units 1,000 to 2,000 soldiers will be selected for study.

3. Identification of Specific Risk Factors

The seroepidemiological studies listed above will provide some information on risk factors for the diseases being studied, but the ideal study design for studying multiple risk factors most economically is the case-control study, in which a large number of risk factors are evaluated using a relatively small number of subjects. Patients presenting with a febrile illness at the ROK army hospital in Seoul will be evaluated clinically and serologically, and attempts will be made to isolate infectious agents. Those who can be definitely diagnosed as having one of the diseases of interest will be entered into one of the three case groups. A control group will be selected from other patients in the same hospital. A detailed questionnaire will be completed for each case and each control. This questionnaire will include questions on MOS; unit; travel history; specific activities of the previous months such as overnight patrols, river crossing, digging foxholes or pit latrines, operation in a dusty environment, etc., concentrating on activities which might result in increased exposure to rodent excreta. This study will provide estimates of the relative risk for each of the potential exposures being evaluated.

MATERIALS AND METHODS

A. Epidemiology

1. Farming Study

The purpose of this study is to chronicle the many different activities performed by rice farmers and their workers throughout the tilling, planting, harvesting, and wintering season. An understanding of the activities and practices of these farmers will provide insight into the kinds of risks/exposures (related to leptospirosis, scrub typhus, or

Korean hemorrhagic fever) that farmers may encounter during the course of a year. This study may help in revealing additional risks/exposures to investigate as well as providing insight into the current practices of rice farmers in Korea.

The Epidemiology team went out and enrolled farmers to help participate in this study. Members of the Epidemiology team interviewed farmers about their farming methods and their activities during the off-season. This will be done on a periodic basis throughout the year. The results of this study will be compiled in a folder describing the farmers, their activities, and the growth of the rice plants.

2. Case Control Study of Risk Factors

The purpose of this study is to identify the risk factors associated with leptospirosis, scrub typhus, and Korean hemorrhagic fever among Republic of Korea soldiers admitted to the Capital Armed Forces General Hospital in Seoul, Korea. The study is a retrospective case-control study of ROK soldiers admitted with the presumptive diagnosis of the above mentioned diseases. The microbiology laboratory at USAMRU-ROK provided serologic support to confirm the case diagnoses. A questionnaire was constructed to facilitate and make consistent the interview process for these soldiers. The questions consist of background demographic data (such as their age, MOS, unit, etc.). The soldiers are then asked the number of different residences that they have been in over the past 2 months. For each of the residences that they were in, the soldiers were asked what types of dwellings they lived in, the types of activities that they performed, and whether or not they had contact with water, dust, rodents or insects, as well as other questions associated with risk factors for the diseases under study.

To control for any potential confounders, controls for the study were randomly taken from ROK soldiers admitted to the same hospital on the same date as the admission date of each case, plus or minus 5 days. The interviewers (nurse epidemiologists) were trained to be consistent in their interviewing process. The interviewees are brought to them without the interviewers knowing whether the soldiers are cases or controls. After the interview, the nurse epidemiologists and an epidemiology supervisor reviewed the interviews for completeness, discrepancies or inconsistencies.

Blood and urine are being collected from symptomatic patients at the Capital Armed Forces General Hospital and the 121 EVAC Hospital under protocols approved by Korean and U.S. human use review committees. Additional data relating to risk factors such as age, sex, occupation, exposure to rodents, area of residence, and other behavioral and demographic factors will be needed.

Univariate analysis was performed to calculate odds ratios for each of the risk factors and their 95% confidence intervals. Stratification by different risk factors to control for potential confounders will be accomplished using the Mantel Haenszel method to obtain odds ratios and their 95% confidence intervals. A check will also be made to fit the data into a multiple logistic regression model once all data collection is accomplished at the end of 2 years.

3. Blood specimens from civilian hospitals throughout Korea were submitted to the Institute for Viral Diseases for diagnosis of suspected Korean hemorrhagic fever. These specimens were also tested for *R. typhi*, *R. tsutsugamushi*, *R. siberica* and leptospirosis. Although not part of a formal study, the information gained may provide valuable clues to the spatial and temporal distribution of human pathogens in the Republic of Korea.

4. Serosurvey of ROK Army Personnel

We propose to conduct a serosurvey of ROK Army personnel to determine point prevalence rates (for specific geographical areas and specific units) for the above-mentioned diseases.

B. Field Ecology and Microbiology

1. Survey Area

- a. ROK - A geozoological survey extending throughout the ROK which samples geographical areas in a 50 km grid has been started (Figure 1). This study will attempt to determine the geographical distribution of leptospirosis, rickettsial diseases and CTF virus. The mammal populations were identified and enumerated. The seroprevalence rate was also determined for each rickettsial disease agent and attempts were made, and will continue to be made, to isolate the disease agents of interest. Relevant ecological parameters such as habitat were also recorded.

Urban rat surveillance for *L. interrogans* will begin this winter in major cities throughout Korea.

- b. Okinawa - One field trip to U.S. Marine Corps bases in Okinawa was conducted to assist the U.S. Navy in investigating an outbreak of leptospirosis and to provide field training in leptospirosis to the ecology field team members during the winter months when there were no cases of leptospirosis in Korea. Two general areas were sampled: the Northern training area and the Central training area.

2. Collection of Field Mammals

Field and house rodents were captured by means of baited Sherman and Tomahawk traps (1,2). The rodents were identified to species and bled under CO₂ anesthesia. Serum was collected for antibody titration to leptospira and scrub typhus and Colorado tick fever (CTF). Tissue samples for isolation of scrub typhus and leptospirosis included lung, kidneys, liver, spleen and lymph node. These are currently stored at - 70C. All rodent ectoparasites were collected for identification. Mites were placed in distilled water for transportation to the laboratory for processing. Ticks were placed in ventilated vials until processed.

3. Specimens from Domestic Animals for Isolation of Leptospira

Limited isolation attempts for leptospira in swine have been done in Pyongtaek by surveying the local slaughter house. Kidney samples from slaughtered animals were cultured for leptospira. More intensive slaughter house and serological surveys will be conducted during the winter months when field surveys of rodents are limited by weather.

4. Specimens from Patients

Blood urine or necropsy tissue were collected from acute phase patients for isolation of virus, leptospira, or rickettsia and sera were collected from suspected leptospira, CTF, or rickettsial patients for serodiagnosis. Larger amounts of hyperimmune convalescent serum were collected when possible for experimental purposes.

5. Virus Isolation

Details of techniques for isolation of CTF and related viruses in suckling mice and Vero cells have been described previously (3).

6. Leptospiral Isolation

Blood, urine or tissue samples were triturated and inoculated into EMJH media as described previously (4). All positive cultures are identified by standard serological techniques and representative cultures have been sent to the WHO Leptospirosis Laboratory at CDC.

7. Rickettsial Isolation

The isolation of scrub typhus from blood, tissue and chigger samples in ICR adult mice has been previously detailed (5). Isolates were identified by standard serological techniques.

8. Serological Tests for Leptospiral Antibodies

An ELISA for leptospiral IgG and IgM antibodies developed at USAMRIID is being used for serological tests in humans. Since this test is relatively new and the prevalence of different leptospiral serovars, besides Mwoyola is presently unknown, a microagglutination test (MAT) (4) is also used in conjunction with the ELISA. Representative serovars from each serogroup are being used. As more serovars are isolated from human and domestic animals studies they will be added to the MAT test to provide a more relevant test.

All serological studies in domestic animals will be done by MAT until the ELISA test is developed for these species.

9. Serological Tests for Scrub Typhus Antibodies

All serology utilizes established IFA techniques (6).

10. Serological Tests for Murine Typhus and Spotted Fever Antibodies

R. typhi and *R. sibirica* antigens were prepared in yolk sac and micro-immunofluorescent antibody technique were used for antibody titration (7).

11. Serological Tests for CTF like Agent

Serological testing for CTF-like agents will be done by IFAT (8) and confirmed by plaque reduction neutralization (PRNT).

RESULTS

A. Laboratory Setup

The first members of USAMRU-ROK, the commander and the NCO, arrived in the Republic of Korea in September, 1988. A data manager, driver and temporary secretary were hired and trained, and visits were made to the endemic areas. Initial contacts were made with Korean civilian scientists and with key members of the U.S. and the ROK military, and the foundation for future research projects was established. A permanent site for the laboratory was renovated and occupied in December 1988.

Between September 1988 and the early Spring of 1989, the equipment and supplies for the new laboratory were shipped from the U.S. to Korea, received, inventoried, stored and installed.

Most of the laboratory staff was hired in April and May of 1989, and their training occupied most of the summer months. Because of several construction delays the BL2 laboratory was not ready for use until July of 1989 (see Figure 2). The BL3 laboratory was ready for occupancy in April of 1990. These delays affected the training of the research staff especially in those techniques requiring the BL3 areas.

B. Time Constraints

Due to the construction delays and subsequent training delays most studies were not started until late into the epidemic periods for both leptospirosis and scrub typhus.

C. Epidemiology

1. Farming Study

a. General

Three farmers residing in Kyunggi-do, Pyongtaek-kun were observed and interviewed on June 14, 1989. Observation of farming activities and practices, follow-up interviews, and pictures were obtained every 2 to 4 weeks for one planting season. There are some slight variations in farming activities and methods based on the size of the farm and on the equipment a farmer can afford. The following describes the activities of one of the farmers. The farming activities can be divided into different seasons.

b. The Spring season runs from March through May. Preparation for sowing begins around late March to early April

(1) Preparation of seeds

The rice seeds are soaked in salty water to select healthy rice seeds and then soaked in an antiseptic solution for 10 days to disinfect the seeds. The seeds are then taken from disinfectant solution and placed in a warm area (30-32C) for 2-3 days for sprouting to occur. For machine transplantation, the sprouts must be at least 1 mm long. For hand transplantation, the sprouts must be at least 2-3 mm long.

(2) Cultivation of the seed-bed sprouts

The selected field is leveled and watered for 1-2 days before seeding. Seed-beds are placed in the field and allowed to grow for 30-33 days until transplantation begins (mid to late May). A greenhouse is constructed over the growing seed-beds to maintain proper temperature and humidity.

(3) Rice transplantation

Rice fields are leveled by using a power tiller or tractor. The fields are then plowed and watered 2-3 days before transplantation. Machine transplantation of rice begins around mid to late May.

- c. The Summer season runs from June through August. The following activities predominate at this time

(1) Weeding

Weeds are pulled out manually or with a hoe. A powdered herbicide is sprinkled about 10 days after rice transplantation. Grass on the bank is cut using a sickle. The bank is also sprayed with herbicide using a power sprayer.

(2) Fertilization

Mid-term fertilization occurs about 15 days after rice transplantation using a chemical composite fertilizer ('21-17-17') sprinkled by hand. Third-term fertilization occurs about mid August, just before the sprouting of rice ears.

(3) Insecticide use

Insecticides for Rice Plant Fever and E.H.M. are employed using a power sprayer. The insecticide is sprayed ever 10-15 days (once before the rainy season and intermittently during the rainy season).

- d. The Autumn season runs from September through November and involves the following activities

- (1) Weeding is the main activity during this period and is done with sickles or knives. The weeds are gathered and

burned.

(2) Draining of the rice field--the bank is opened with shovels and the water from the rice field is drained before mid September.

(3) Plants are bunched--4 plants to a bunch. This allows them to stand together better and increases the yield per plant.

(4) Insecticides and herbicides are administered periodically with a power sprayer.

(5) Rice harvesting

Rice harvesting is done by a combine. The rice grains are automatically cut, threshed and packed into bags.

Drying the grain may be accomplished by one of two methods:

Using a grain dryer, the grain is dried at a temperature of 35-45 C.

Using a storage room the grain is dried using natural ventilation. This takes about 7-10 days.

(6) Preparing the rice fields for next year

The rice fields are covered with compost made from cut rice straws to fertilize the soil.

2. Case Control Study of Risk Factors

a. General

The relatively short length of time from the establishment of USAMRU-ROK precludes us from having any detailed epidemiologic or statistical studies on the risk factors for Leptospirosis and Scrub typhus. Table 1 and table 2 summarize some of the demographic characteristics of patients who have been diagnosed with leptospirosis or scrub typhus.

b. Leptospirosis

All six leptospirosis patients were young male ROK soldiers. All were exposed to the field before they became sick, and four of the six had an MOS in the combat arms. They unanimously

reported a risk factor of exposure to ground water or mud. Five had scratched skin or abrasions and were then exposed to ground water or mud. They report performing the following activities during their exposure: two were exposed while creeping/crawling, two while crossing a stream, and two while helping with rice harvesting. Reporting on the locations of their exposures: three were exposed in rice fields, two at Field Training Camp, and two at Base Camp. Reporting on the extent of their exposure: four had wet clothes and two had their arms and feet wet.

c. Scrub Typhus

All of the confirmed scrub typhus patients were young males. Three of the four had an MOS in the combat arms. Three reported being bitten by insects but not by mosquitoes. Three reported sitting in the dirt and two reported both sitting and lying on the grass.

d. Future Plans

We are considering possibly expanding the farming study in the future to include farmers from different geographical areas as well as farmers that grow crops other than rice. This would be helpful for the development of questionnaires for future case control studies delineating risk factors or activities specific for the above mentioned diseases for workers in the agricultural industry.

3. Diagnostic Serology on Specimens Submitted by Civilian Hospitals in Korea in 1990

The total no. of serum specimens from civilian patients with hemorrhagic manifestations examined for Hantaan virus, *R. typhi*, *R. tsutsugamushi*, *R. Siberica* and Leptospirosis at the Institute for Viral Diseases in 1990 was 3,268. There were 464 cases of HFRS, 683 cases of scrub typhus, 198 cases of murine typhus, 49 cases of spotted fever and 140 cases of leptospirosis confirmed serologically among 3,268 civilian suspect hemorrhagic disease patients. The percentage of serologically confirmed patients among clinically suspected patients are shown in Table 3. it is noteworthy that the etiology of 53% of the clinically suspected hemorrhagic disease patients was unknown by the methods employed in the study.

The geographical occurrence of HFRS, murine typhus, scrub typhus, spotted fever and leptospirosis is shown in Table 4. The majority of the hemorrhagic disease patients were identified in Seoul

city, Kyunggi-do, Chungcheongnam-do, Kyungsangnam-do and Jeollanam-do, the northern and southern-western parts of South Korea. Monthly incidence of HFRS, murine typhus, scrub typhus, spotted fever and leptospirosis is shown in Table 5. Outbreaks of HFRS, murine typhus, scrub typhus and leptospirosis occurred in the fall of 1990 during epidemic season of HFRS; however a small no. of patients occur throughout the year.

D. Field Ecology

1. Scrub and Murine Typhus

Table 6 summarizes the results to date on serology for scrub and murine typhus. KHF titers were also performed on each animal and are reported. A total of 1785 IFAT assays were performed (591 for scrub typhus, 590 for murine typhus and 604 for KHF) on 8 different general of indigenous mammals from various sites throughout the ROK. Additionally, a total of 266 leptospirosis cultures were initiated. A 6.8% and 21% scrub typhus infectivity rate was noted for *Apodemus* and *Rattus* respectively. Most of the scrub positive animals were trapped in the Pyongtaek area with a few also trapped in the Unchon area. A very low infectivity rate of murine typhus was observed in all mammals tested.

2. Leptospirosis

Table 7 details the isolation attempts from vertebrate tissues collected by the field ecology team. A total of 4 isolates of leptospira have been made from *Rattus* collected in Seoul (2) and Okinawa (2) and 3 from *Herpestes* from Okinawa. A total of 20 swine samples were also tested from Pyongtaek, and were all culture negative. There were no isolates from 193 *Apodemus* spp. collected and there were also no isolates from the amphibians collected in Okinawa.

3. Colorado Tick Fever

Due to time constraints, serology for CTF on all mammal sera and isolation attempts from tissue for CTF and scrub typhus collected to date will be conducted during the winter months when more time is available.

E. Microbiology

1. General

To date, the primary activities of the Microbiology department

have been in support of studies carried out by the Epidemiology and Field Ecology departments. With the acquisition of field specimens from both animal and human sources, the activities of the lab will shift to more of a research nature as described in the method of approach.

2. Microbiological Support of the Field Ecology Department

Approximately 60% of the work performed by the Microbiology Department during the period covered by this interim report was performed in support of ongoing projects of the Field Ecology Department. The type of support provided can be broadly described as serodiagnostic support.

3. Microbiological Support of the Epidemiology Department

Approximately 30% of the work performed by the Microbiology Department during the period covered by this interim report was performed in support of ongoing projects of the Epidemiology Department. From November 1989 to October 1990 sera from patients in the Capital Armed Forces General Hospital, Seoul, Korea was collected by the Epidemiology Department. These samples were analyzed for IgM and/or IgG titers to leptospira, scrub typhus, and hantaan virus. These results are reported in Table 8. Of the total 105 patients admitted to the KHF ward, 91 patients (86.7 %) possessed serum antibodies to KHF. Two patients did not possess antibodies to KHF, but did serologically react to the scrub typhus agent. This suggests that these patients were in fact scrub typhus cases. Interestingly, 12 of the 105 patients (11.4%), with a clinical presentation of a KHF-like illness, did not possess serum antibodies to leptospirosis, scrub typhus rickettsia, or KHF. This indicates that a significant percentage of KHF-like clinical illness is caused by an as yet unidentified agent, possibly CTF, a CTF-like virus, or an antigenically distinct strain of rickettsia. Studies this winter will attempt to determine if these pathogens are the causative agents of disease in these unexplained illnesses. It should also be noted that of the 89 patients admitted to the internal medicine ward, three were serologically diagnosed as KHF patients. This implies that, in these patients, the early clinical symptoms were not indicative of KHF, and suggests that the early symptomatic manifestation of Hantaan virus infection may vary from patient to patient.

4. Microbiological Support of U.S. Forces in the Pacific

The Department of Microbiology provides diagnostic support to the 121st Evacuation Hospital in Seoul, Korea; U.S. Forces, Korea, and the marines stationed on Okinawa. Most of the diagnostic specimens

submitted are from military members stationed in Korea, or on a training exercise in Korea. Some specimens tested for leptospirosis came from marines stationed on Okinawa who were believed to have acquired their disease there. Between 3 October 1989 and 29 Jan 1991, 85 patients were tested for KHF, of which 12 (14%) were diagnosed as acute KHF; 89 were tested for leptospirosis of which 1 was diagnosed as an acute case and 5 as probable cases; and 73 were tested for scrub typhus, of which 2 were diagnosed as acute cases.

CONCLUSIONS

It must be emphasized that the results reported here are preliminary and are based on animal and human serosurveys that have occurred over a limited amount of time, been performed on a limited number of specimens, been performed in a limited number of sites (mostly in sites in the North and Central portion of the ROK), and may not have occurred during the peak season of activity of a given pathogen. As such, it is difficult to draw conclusions from this data. Whether or not the apparent lack of murine typhus antibodies and leptospirosis isolates in the animals tested suggests that these pathogens are not important potential disease-causing agents remains to be seen. Conversely, the clinical significance of the relative abundance of tick typhus seropositivity also remains to be proven. Our preliminary data suggest that a significant percentage of KHF-like illness is not due to infection by Hantaan virus. As additional samples are accumulated and analyzed, the trends in the data will become more clear. Now that all construction, equipping, and staffing of the laboratory has been completed, efforts can be concentrated fully on data acquisition, analysis, and interpretation.

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Figure 1 Geozoological Survey

LOCATIONS-ROK

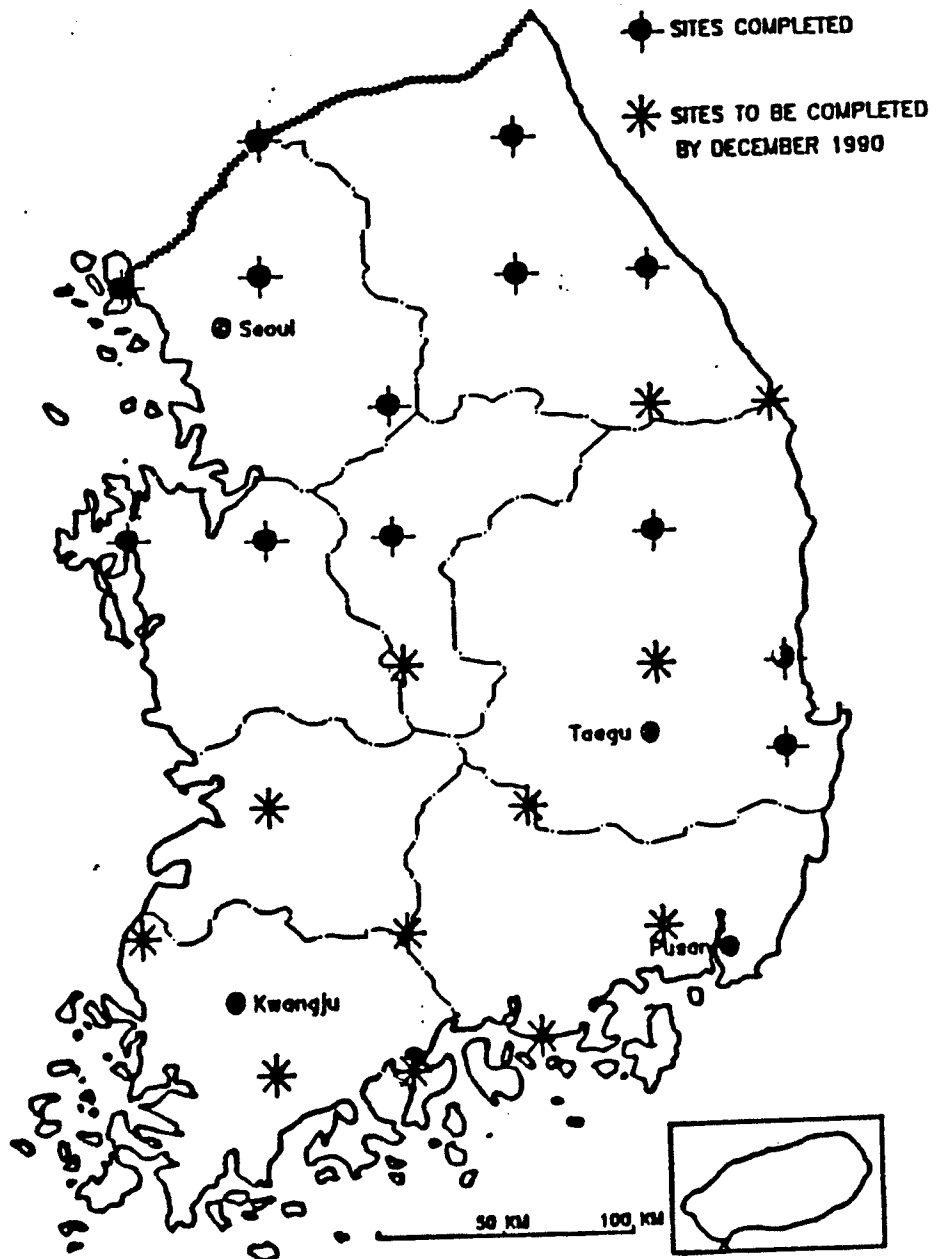
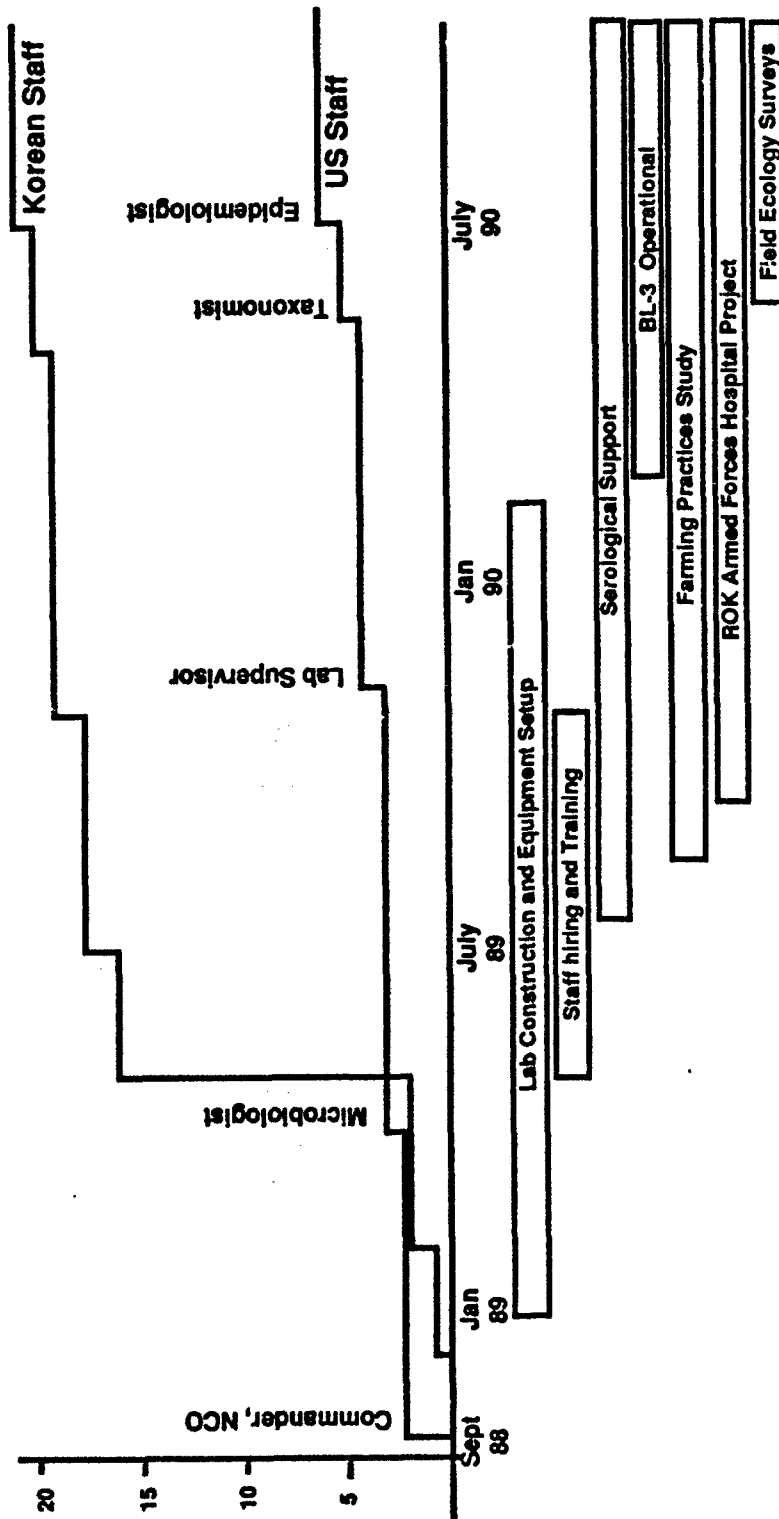


Figure 2

USAMRU-ROK HISTORY



**Table 1. Demographics of Leptospirosis Patients* Identified
at the Capital Armed Forces General Hospital
in Seoul, Korea, 10/89 to 10/90**

<u>Pt.</u>	<u>Age</u>	<u>MOs</u>	<u>Date of Onset</u>	<u>Residences†</u>
1	21	Infantryman	10/23/89	Chungbuk, Jungwon-gun
2	21	Recoilless gunner	09/24/90	Kyonggido, Icheon-gun
3	21	Tel. operator	09/24/90	Kyongbuk, Youngcheon-gun
4	22	Tank crewman	09/26/90	Kyonggido, Icheon-gun
5	21	Wire installer	09/27/90	Kyonggido, Yangpyung-gun
6	21	Infantryman	09/27/90	Kyonggido, Yeoju-gun
				Kyonggido, Yangpyung-gun
				Kyonggido, Yangpyung-gun

*Current as of 28 September 1990

†Locations of residences just prior to date of onset of illness

**Table 2. Demographics of Scrub Typhus Patients Identified
at the Capital Armed Forces General Hospital
in Seoul, Korea, 10/89 to 10/90**

<u>SCRUB TYPHUS</u>				
<u>Pt.</u>	<u>Age</u>	<u>MOs</u>	<u>Date of Onset</u>	<u>Residences†</u>
1	22	Infantryman	10/21/89	Kangwondo, Inje-gun
2	22	Infantryman	05/01/90	Kyonggido, Pocheon-gun
3	21	Dispensary aide	09/18/90	Kangwondo, Hongcheon-gun
4	22	Machine gunner	10/09/90	Kangwondo, Chulwon-gun

+Locations of residences just prior to date of onset of illness

Table 3. Total number of patients with hemorrhagic fever with renal syndrome (HFRS), murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis diagnosed serologically among civilian suspect hemorrhagic fever patients in Korea, 1990.

Disease	No. of patients	
	No. of serum tested	
HFRS	464/3,268	(14%)
Murine typhus	198/3,268	(6%)
Scrub typhus	683/3,268	(21%)
SFG rickettsiosis	49/3,268	(2%)
Leptospirosis	140/3,268	(4%)
Unknown	1,734/3,268	(53%)

Table 4. Geographical distribution of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis among civilian suspect hemorrhagic fever patients in Korea, 1990.

Name of province	No. of case				
	HFRS	murine typhus	scrub typhus	spotted fever	leptospirosis
Seoul city	91	85	108	21	12
Kyunggi-do	161	31	69	14	32
Kangwon-do	12	7	15	0	5
Chungcheongbuk-do	8	5	18	2	5
Chungcheongnam-do	79	21	72	2	8
Kyungsangbuk-do	22	3	25	0	2
Kyungsangnam-do	15	13	110	3	7
Jeollabuk-do	8	2	20	2	4
Jeollanam-do	50	23	193	4	50
Cheju-do	0	2	4	0	0
Unknown	18	6	49	1	15
Total	464	198	683	49	140

Table 5. Monthly incidence of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis among civilian suspect hemorrhagic fever patients in Korea, 1990.

Month	No. of patient/no. of serum tested					
	HFRS	murine typhus	scrub typhus	spotted fever	leptospirosis	unknown
1	25/108	15/108	0/108	7/108	0/108	61/108
2	5/57	10/57	2/57	8/57	0/57	32/57
3	10/103	29/103	3/103	0/103	1/103	60/103
4	11/117	13/117	1/117	0/117	1/117	91/117
5	10/117	9/117	1/117	0/117	1/117	96/117
6	26/128	6/128	14/128	6/128	0/128	76/128
7	13/103	4/103	12/103	15/103	3/103	56/103
8	12/170	8/170	31/170	2/170	11/170	164/170
9	20/237	14/237	3/237	6/237	35/237	159/237
10	63/725	40/725	171/725	1/725	72/725	378/725
11	178/1093	33/1093	406/1093	1/1093	16/1093	459/1093
12	91/310	17/310	39/310	3/310	0/310	160/310
Total	464/3268 (14%)	198/3268 (6%)	683/3268 (21%)	49/3268 (2%)	140/3268 (4%)	1734/3268 (53%)

Table 6. Results of mammalian serology tests conducted by the
microbiology department in support of
USAMRU-ROK ecology studies

Genus	KHF IFAT	Scrub typhus IFAT	Murine typhus IFAT
<i>Apodemus</i>	58/580	34/542	1/541
<i>Rattus</i>	0/23	4/19	0/19
<i>Mus</i>	0/10	0/9	0/9
<i>Herpestes</i>	nt**	nt	nt
<i>Amphibians</i>	nt	nt	nt
Others*	0/21	2/21	0/21
Total	604	591	590
* Other genera tested include <i>Shrews, Microtus, and Tamias.</i>			
** nt=Not tested			

**Table 7. Results of mammalian leptospirosis cultures conducted
by the microbiology department in support of
USAMRU-ROK ecology studies**

Apodemus	Rattus	Genus mus	Herpestes	Amphibians
0/193	4/40	nt*	3/7	0/26

* not tested

Table 8. Results of serological tests performed on soldiers hospitalized at the Capital Armed Forces General Hospital (6 NOV 1989 to 10 OCT 1990)

Hospital Ward	Serological Diagnosis				Total No. of Patients
	KHF	Lepto	Scrub typhus	Undetermined	
KHF	91	0	2*	12	105
Internal Medicine	3	3	1	82	89
Total	94	3	3	94	194

* *R. tsutsugamushi* was isolated from one patient